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STRUCTURES OF THE BLOOD-BRAIN BARRIER THAT CONFERS ITS IMPERMEABILITY AND INNOVATIONS IN DRUG DELIVERY TO THE CENTRAL NERVOUS SYSTEM

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ABSTRACT

The blood-brain barrier (BBB) is a critical regulatory structure that maintains neural homeostasis by selectively controlling the substances that enter the central nervous system. While this impermeability protects the brain from harmful agents, it poses a formidable challenge for delivering drugs to treat neurological disorders such as Alzheimer's, Parkinson's, and brain cancers. This review analyzes the structural components contributing to the BBB's restrictive nature, including endothelial cells with tight junctions, astrocytic end-feet, and pericytes, and highlights their implications for drug delivery. Two primary approaches to overcoming the BBB are examined: invasive techniques such as hyperosmotic agents and inflammatory mediators, and non-invasive methods including liposomal carriers and intranasal delivery.

While invasive techniques can effectively enhance BBB permeability, they carry risks of adverse side effects, such as neuronal damage or exposure to harmful substances. Among non-invasive strategies, liposomal drug delivery systems show promise due to their ability to encapsulate both hydrophilic and hydrophobic drugs. However, these systems still face challenges due to limitations in current molecular drug design. Liposomes require fine-tuning to meet the BBB's molecular weight and hydrogen bonding restrictions, and advancements in medicinal chemistry are essential to develop effective carrier systems that can efficiently target the CNS without compromising safety.

Furthermore, discrepancies in findings across studies, attributed to methodological and species differences, underscore the complexity of achieving consistent results. This review emphasizes the necessity for innovative research to refine these methods and mitigate their shortcomings, with the ultimate goal of enabling safe and effective treatment for currently intractable neurological diseases.

KEYWORDS: Blood-Brain Barrier, Drug Delivery, Neurological Disorders, Endothelial Cells, Tight Junctions, Nanoparticles

INTRODUCTION

The blood brain barrier (BBB) is a highly selective structure in the central nervous system (CNS), wedged between the capillaries—that carries the essential nutrients for the brain—and the brain tissue itself. Its restrictive traits allow it to maintain the brain's homeostasis by regulating what enters and exits, protects the brain's microenvironment from harmful substances, and supplies the brian with necessary nutrients to operate efficiently (Pardridge, 2005). However, due to the special unique traits that make up the BBB's restrictive nature, it also limits the entry of neurotherapeutics for brain diseases such as Alzheimer's, Parkinson Disease, as well as brain cancer. Therefore, many prominent brain diseases are left uncured today due to this impassable barrier and current research efforts are focused on methods to bypass the BBB in order to successfully deliver drugs to the site of injury inside the brain. While its structure, function, and impermeability to the types of molecules is well understood, current research still grapples with the challenge of delivering drugs across this barrier without compromising its protective function.

Only 98% of all small molecule drugs do not cross the BBB (Pardridge, 2005). The unique composition of the BBB only permits lipid-soluble drugs that have a molecular weight ¡ 400 Da and have ¡ 8 hydrogen bonds. If the drug does not follow these characteristics, it would not enter the BBB (Wu et al., 2023).

Previous studies have elucidated the BBB's selective permeability, governed by tight junctions, endothelial cells, and other physical and biochemical properties. The lining of the capillaries are made of endothelial cells (ECs), however, unlike other types of periphery ECs, the BBB's ECs contain unique characteristics that contribute to the BBB's impermeability: (i) a high electrical resistance due to the tight junctions restricting its paracellular flux, (ii) a higher mitochondrial volume inside the ECs which makes transporting energy unnecessary, (iii) and no fenestrations which greatly limits the amount of molecules that can diffuse freely across the blood and brain tissue (Tajes et al.). Furthermore, the ECs are tightly sealed by tight junctions (TJ) that seal any gaps in the EC along with adherent junctions (AJ) that are responsible for the initiation, cell-cell contact, and adhesion of ECs. The BBB's ECs are additionally enwrapped with pericytes that are responsible for regulating vascular tone, along with the end-feet of astrocytes that forms as an additional restrictive layer. Hence, all of those characteristics collectively

act as physical barriers that prevent the free flow of substances and the BBB's high selectivity.

BBB drug delivery solutions can be categorized into two types of methods: BBB disruption (direct technique), and indirect techniques. We know that methods such as BBB disruption, lipidization of drugs, and nanoparticle-based carriers can improve delivery to the central nervous system. Out of the most prominent methods, indirect approaches, particularly liposomal cargos, hold the most potential for effective drug delivery with the least adverse side effects. However, these strategies are fraught with limitations, including variability in efficacy, off-target effects, and the risks of neuronal damage or systemic toxicity.

Furthermore, key questions persist: How can drug carriers be optimized to meet the molecular and structural requirements for BBB penetration? What mechanisms cause the discrepancies observed in BBB disruption studies across species and methodologies to ensure accurate analysis of the efficacy of BBB drug delivery approaches? This review seeks to address these gaps by critically evaluating current invasive and non-invasive techniques for BBB drug delivery, exploring their mechanisms, outcomes, and challenges. Employing a synthesis of existing research, the study identifies patterns in experimental design and highlights areas requiring further exploration, such as refining molecular design and enhancing drug-carrier compatibility.

METHOD SECTION

For this research, I gathered sources that discuss a solution for BBB delivery and the structure of the BBB. I applied this exclusion and inclusion criteria while I was finding sources to ensure I found the most useful information that is relevant to my research question. I used Google Scholar to find my sources and only used articles. Search terms I used were genetic engineering, gene therapy, gene therapy cancer, then shifted to blood-brain barrier, blood-brain barrier permeability, blood-brain barrier structure, and blood-brain barrier delivery. From this, I found 19 articles and concluded with 7 articles to use for my research paper.

Exclusion Criteria	Inclusion Criteria
• Articles that do not focus on the blood-brain barrier itself or its direct involvement in drug delivery, such as those dealing solely with diseases that are not related to BBB disruption. • Studies that discuss neurological diseases or treatments without any mention of BBB drug delivery challenges, solutions, or potential therapies. • Articles that do not provide results from in vitro, animal model, or clinical trials testing BBB drug delivery approaches. • Exclude articles that are not published in English, unless they are accompanied by an English translation or sufficient summary that can be used for analysis.	• Research that specifically addresses methods or technologies for overcoming the BBB to deliver neurotherapeutics or drug carriers. • Study explores both invasive (e.g., BBB Disruption) and non-invasive (e.g., nanoparticle carriers, liposomal delivery) strategies for improving BBB drug delivery efficiency. • Provides information about the physiological and biochemical structure as well as restrictive mechanisms of the BBB, including the role of endothelial cells, tight junctions, astrocytes, and pericytes. • Research focusing on the molecular composition of the BBB (lipid solubility, molecular weight, hydrogen bonding, etc.) that influences the passage of drugs or therapeutic agents across the barrier. • Discusses the cellular and molecular interactions at the BBB, including the role of transporters, receptors, and enzymes • Studies that evaluate the efficacy, flaws, safety, and outcomes of BBB drug delivery techniques.

Almost all of the BBB delivery solutions discussed in these articles were experimented on a population of animals, namely mice, frogs, pigs, and cats with a variety types of brain injury, and sometimes volunteering ill-patients. However, in these sources, they don't specify the gender, age or personal information of its population, and hardly mention the feasibility of these methods that are related to cost. Also, they do not specify geographically where these studies were conducted, but based on the authors' background information, it suggests that some of the research that they performed was predominantly from the US and China. When analyzing results, the researchers examine the effects of their methods on the structures of the BBB and changes in reactions that suggest a disruption in the BBB. Moreover, when some of the sources are reviewing multiple kinds of BBB delivery solutions, they mention and explain that the discrepancies and diversities found in multiple researchers' results obtained from certain methods may be subjected to regional, methodological, and species differences. (Regulation of Blood–Brain Barrier Permeability, 2013). The outcomes measured in these sources focused on multiple targets, such as the reaction of the BBB's structures in response to particular stimulus, measuring the maximum molecular weight and other requirements of a drug in order to be permitted into the BBB, as well as the negative or harmful side effects from a method; hence all of these results were measured objectively.

BBB Disruption

BBB disruption is a method that directly targets weakening the BBB itself to make it more permeable for drugs to pass through. The two most prominent methods are using hyperosmotic agents, and inflammatory mediators.

Inflammatory Mediators

A study reports that the brain's endothelial cells responses

with an increase in its permeability towards inflammatory mediators, which are chemicals released by the immune system to induce inflammation to protect the body from infection; hence suggesting that one of the brain's qualities is to modulate its permeability in response to these triggering agents (N. Joan Abbott, 2000). Certain inflammatory agents can promote a reduction in arteriolar and increase in venular tone, which altogether stretches the TJs within the capillaries, effectively increasing the opening of the paracellular pathway for drugs to pass through. One of frequently-tested inflammatory agents is Bradykinin (Bk). In rat and frog pial microvessels, researchers discovered that Bk causes a reversible reduction in the transendothelial electrical resistance in both animals. Thus, these results suggest that the reduction in the transendothelial electrical resistance is derived from an opening of tight junctions caused by Bk. Investigators have used intravital fluorescence microscopy to demonstrate that when bradykinin is topically applied, it specifically enhances the leakage of fluorescent tracer from cerebral blood vessels by activating B2 receptors. This increase in permeability selectively affects the transport of smaller molecules compared to larger ones, indicating that bradykinin induces endothelial cell contraction to create a pathway for molecule movement across the blood-brain barrier. This concept is further supported by studies suggesting that bradykinin triggers an elevation in intracellular calcium levels within endothelial cells, which is known to increase the permeability of cerebral venules. However, additional investigations are required to accurately ascertain the specific cellular mechanisms through which bradykinin leads to an increase in blood-brain barrier permeability. Gaining a comprehensive understanding of the pathways activated by bradykinin could offer valuable insights for developing treatments for individuals experiencing cerebrovascular trauma. Previous studies have utilized various techniques to investigate the impact of histamine on blood-brain barrier permeability to both large and small molecules. These investigations have demonstrated that histamine infusion into the artery increases the permeability of the blood-brain barrier to molecules of different sizes, such as sucrose, alpha-aminoisobutyric acid, and horseradish peroxidase. Similarly, topical application of histamine in vivo has shown to dose-dependently increase the permeability of the blood-brain barrier to tracers with varying molecular weights. Moreover, histamine application has been found to reduce the electrical resistance of cerebral arterioles and venules, thereby increasing the blood-brain barrier permeability. Likewise, Butt and Jones (1992) and Butt (1995) discovered that histamine caused a reversible reduction in transendothelial electrical resistance of pial vessels. Furthermore, the administration of histamine resulted in a comparable level of opening, suggesting that the first phase of opening seemed to be attributed to the release of free radicals. When histamine at low concentrations (ranging from 5 nM to 5 mM) was applied, acting on H2 receptors, it enhanced permeability. However, higher concentrations of histamine (ranging from 50 to 5 mM) reduced permeability by interacting with H1 receptors. Numerous investigations involving in situ brain microvessels have demonstrated that the application of histamine, whether to the luminal (inner) or brain side, resulted in a relatively indiscriminate rise in permeability. This was observed by using dyes with molecular weights of up to 150,000 Da. However, contrasting findings have been reported in other studies, implying potential variances attributable to species variations and methodological disparities. According to Matsukado et al, in vivo and in vitro studies confirmed that inflammatory agents such as bradykinin, histamine and other molecules such as solvents, stabilizers, or adjuvants increase the BBB's permeability. These molecules integrate with the phosphorylation or dephosphorylation of the tight junctional complexes, which relaxes the tight cell-to-cell adhesion. This was demonstrated when Alkermes Incorporation created a synthetic bradykinin called Cereport (RMP-7). Cereport functions by binding to the bradykinin B2 receptor in cerebrovascular tissue, leading to an elevation in cyclic GMP levels. This process results in the temporary disruption of tight junctions in brain endothelial cells and an increase in brain permeability (Tajes et al.). Furthermore, research efforts have also explored the neuronal effects of the highly infectious disease of meningitis on the BBB. One of the significant factors contributing to the adverse neurological outcomes in bacterial meningitis is the inflammatory response triggered by endotoxin, which leads to the disruption of the blood-brain barrier (BBB). Scientists investigated the effects of the bacteria's lipopolysaccharide properties, a major component of its outer membrane that triggers an inflammatory response. The results demonstrated that the effects of lipopolysaccharide on the cerebral circulation enhanced BBB permeability (Regulation of Blood–Brain Barrier Permeability, 2013). This was verified when a clearance of FITC-dextran 10K was observed, a fluorescent marker to evaluate the passage of molecules across the vasculature; this indicates whether the drugs successfully traversed across the BBB by a given inflammatory agent, in other words, a BBB disruption. It can also help evaluate the effectiveness of a specific inflammatory substance on the permeability of the BBB. Furthermore, other factors involving BBB disruption that were observed were the activations of various cellular pathways, such as nitric oxide synthase, activation of cyclooxygenase, and/or activation of matrix metalloproteinases. Although inflicting inflammation has effectively led to BBB disruption, there are still many unknown and incomprehension of other involving factors such as the role of nitric oxide, a frequently resurfacing element in BBB disruption. As well as many research discrepancies due to methodological and species differences fail to reinforce a common trend in how these mediators operate. However, with further understanding of these little-known transporters and activated molecules, this technique can serve to be a prospectively efficient method in BBB disruption.

Osmotic Disruption of the BBB

Hyperosmotic solutions can be used to temporarily and reversibly open the BBB's tight junctions. Hyperosmolar solutions are made of highly concentrated saccharide solutions are infused into the carotid artery which possesses a direct pathway to the CNS (Tajes et al.). The solution's great hypertonicity and high osmotic pressure forces the water within endothelial cells to withdraw, which shrinks them in size; thus creating a modest opening within the tight junctions for water-soluble drugs—that cannot diffuse across

the BBB—to flow through (Regulation of Blood–Brain Barrier Permeability, 2013). One experiment proved this method by using an intracarotid injection of osmotic solutions which led to the increase of the BBB's permeability. Also, hyperosmolar solutions induce a substantial increase of intracellular calcium concentrations within bovine brain capillaries inside endothelial cells which induce second messenger systems to increase the permeability of the BBB. However, it's important to note that other unknown mechanisms that were observed during these studies may have also contributed to the opening of the BBB. Conversely, in an anticancer therapy experiment, mannitol was used as a hyperosmolar agent to help deliver cyclophosphamide, procarbazine and methotrexate. As a result, the rat brain revealed that hypertonic infusion of mannitol created neuronal damage, alteration in glucose uptake, expression of heat shock proteins and micro embolisms. (Tajes et al.). Therefore, more research is needed to investigate these other cellular mechanisms that account for the opening of the BBB when applying hyperosmolar solutions as well as ways to reduce its harmful side effects.

Although BBB disruption techniques' can successfully open the BBB due to its invasiveness, this attribute also leads to potential adverse effects. On the positive side, it allows for increased permeability of the BBB and enables a wide diverse range of drugs, proteins, peptides, genetic materials, and drug delivery systems like liposomes or nanoparticles to pass through without requiring the modification of their chemical structure. Conversely though, opening the TJs may facilitate the passage of other molecules and unwanted substances, including pathogens as the paracellular route is not selective enough to exclude toxins and undesirable molecules from entering the CNS. Therefore, it is crucial to have control over the timing and duration of the reversible TJ opening, and ensure that frequent stimulation does not negatively impact the BBB or brain conditions. By achieving this control, this approach can offer both effectiveness and safety in brain therapy.

Physical Stimuli

The energy conversion of external stimuli-mediated pathways have been explored as a viable way of BBB disruption (Wu et al., 2023). For example, light could help temporarily and irreversibly open the BBB. In 1990, Eggert et al. conducted research on the effects of Nd:YAG laser irradiation. Their study revealed that the laser treatment resulted in the immediate breakdown of the blood-brain barrier (BBB) due to damage to the microvessels within the brain. According to Eggert et al, the dysfunction of the BBB reached its peak approximately two hours after the irradiation and lasted for about 24 hours. However, the extent of this process is influenced by factors such as the intensity of the laser power, duration of irradiation, and the distance between the laser source and the targeted area. All of these factors contribute to the increase in temperature. Fortunately though, technology developed for non-contact measurement helps monitor temperature elevation, preventing its effects. This technology was used during a study when researchers used near-infrared light (NIR)—an effective method for its deep tissue penetration—irradiation for BBB permeability regulation and the head temperature was kept lower than 43 °C. The NIR temporarily opens the BBB by reducing the electrical resistance of the transendothelial cells which could recover after 10 minutes. In a study conducted in 2021, Qin et al. employed a technique involving the use of targeted nanoparticles combined with the BV11 antibody to modulate the blood-brain barrier (BBB). Their findings demonstrated that upon light stimulation of the BV11-modified nanoparticles, the tight junctions of the BBB opened, enabling the passage of particles such as macromolecules and viruses. Another common and non-invasive external-stimuli is ultrasound that focuses acoustic energy deeply into the body's tissues that releases mechanical or elastic vibrations in a frequency of 18–20 kHz with minimal harm to peripheral tissues. In their research, Hynynen et al. presented a method where the introduction of a preformed gas bubble prior to focused ultrasound irradiation allows for the targeted and temporary opening of the BBB without causing immediate harm to neurons or delayed ischemia. This innovative approach not only limits the effects of ultrasound to the vasculature but also reduces the power required to achieve BBB opening. As a result, this technique enables ultrasound application through the intact skull while minimizing potential risks. Additionally, one of the transient ultrasound approaches plausible for BBB opening is thermal, which causes a mild hyperthermia (Tajes et al.). Considering that hyperthermia has been shown to enhance the permeability of membranes, selectively inducing thermal opening of the blood-brain barrier (BBB) is being considered as a potential approach to improve the delivery of drugs to the CNS. The key advantage of this technique is its site-specific nature, which also reduces the risks associated with a generalized opening of the BBB, making this technique an efficient non-invasive approach in CNS drug delivery. In MRI-guided focused ultrasound study, this technique facilitated the "delivery of anti-Abeta antibody, BAM-10 to Abeta plaques in targeted cortical areas following intravenous injection in a mouse AD model." The decrease in Abeta levels following the treatment indicated that the transportation mechanisms facilitated by focused ultrasound may include "transcytosis, transendothelial openings, fenestration, channel formation, partial opening of tight junctions," and passage through the injured endothelium (Chen & Liu, 2012). In recent years, ultrasound has garnered considerable attention as a physical approach to temporarily open the BBB and improve drug delivery to the brain. While the exact mechanisms by which ultrasound facilitates drug transport and its long-term effects on tissue are not yet fully understood, the use of MRI-guided focused ultrasound offers several advantages. It allows for both diagnosis and treatment simultaneously and enables the enhancement of not only small molecules but also larger compounds such as proteins, liposomes, and nanoparticles. It is imperative to advance the technology to enable the repeated utilization of ultrasound bursts without causing prolonged harm to the tissue and the BBB.

Non-invasive Techniques

Methods that focus on the delivery of the drug across the BBB instead of directly weakening the BBB are considered non-invasive techniques. Two exemplar and prevalently-discussed methods are through increasing the drug's lipophilicity or

encapsulate it inside a lipid carrier. As well as administering the drug through the nose, which possesses a direct pathway from the nasal to the brain.

Lipidization of Drugs

Scientists use medicinal chemistry techniques to reformulate water-soluble drugs into lipid-soluble ones to increase the drug's lipid composition or reduce its ability to bind with hydrogen. These attributes would allow the drug to passively diffuse across the BBB because the BBB's cellular membrane is made of a lipid bilayer, allowing lipid-soluble drugs to integrate across the membrane's similar lipid properties and then passively diffuse through the BBB(Tajes et al.). However, the real-life application of this method is difficult to perform; hence, there isn't a successful instance of reformulating a water-soluble drug into a lipid-soluble drug in pharmacologically significant amounts (Pardridge, *Blood–Brain Barrier Delivery*). Moreover, scientists have created a fundamental guideline to determine whether or not a drug can cross the BBB via lipid-mediated free diffusion in pharmacologically significant amounts based on two attributes: the number of hydrogen bonds and the drug's molecular weight.

BOX 1

Two-step method for prediction of whether a small-molecule drug crosses the BBB via lipid-mediated free diffusion

Step 1: determine molecular weight (MW) of drug
Step 2: determine H-bonding based on drug chemical structure
H-bonding rules:
4 H-bonds for each terminal amide group
3 H-bonds for each internal amide, primary amino group or carboxyl group
2 H-bonds for each hydroxyl group
1 H-bond for each ether or carbonyl group
Add total H-bonds formed between drug and solvent water

Parameter	Unrestricted BBB transport	Restricted BBB transport
MW	<400 Da	>400 Da
Total H-bonding	<8	>8

If the MW of the drug is >400 Da and/or the drug forms eight or more H-bonds, then the drug is probably a poor CNS-penetrating molecule.

Certain functional groups abort BBB transport, for example:
Quaternary ammonium group
More than one carboxyl group

Figure 1: Two-step method for predicting small molecule drug permeability across the BBB. This illustrates a systematic approach for assessing whether a small molecule drug can cross the BBB via lipid-mediated free diffusion. From Blood–brain barrier delivery, by William M. Pardridge 2007

If the number of hydrogen bonds that the drug forms with water is more than 8, it is less likely to be able to diffuse freely across the BBB under pharmacologically significant amounts; therefore, scientists aim to reduce the drug's ability to bind with hydrogen. Likewise, if the drug's molecular weight is greater than 400 Da, it would also be less likely to diffuse across the BBB. Nonetheless, increased lipophilicity of a drug simultaneously increases its uptake in peripheral tissues; consequently decreasing its AUC. Therefore, the field of medicinal chemistry is not yet advanced enough to be able to execute lipid-mediated transport drugs that meet these conditions.

Alternatively, instead of lipid-mediated transport, research efforts have explored a different delivery method: carrier-mediated transport. Scientists use liposomes; a spherical vesicle composed of a lipid bilayer membrane—resembling the structure of a cell—with a hollow aqueous chamber inside that allows it to encapsulate hydrophobic or hydrophilic drugs; thus making this method versatile as it wouldn't require the lipidization of water-soluble drugs, a difficult method to execute that was mentioned in the earlier paragraph. These artificial lipid-based liposomes act as a vehicle that encapsulates the drug and successfully delivers it across the BBB as its lipid-based properties allow it to freely diffuse across the BBB's lipid bilayer. Therefore, these useful properties make liposomes one of the best solutions for BBB drug delivery. Moreover, past research dedicated to liposomal formulations have been studied more extensively and advanced. Researchers have created functionalized strategies to further develop liposomal drug delivery systems to become more efficient, such as "brain/tumor-targeting delivery, controlled drug release, imaging-guided delivery, etc" (Wu et al.) Furthermore, brain-targeted delivery of liposomes can be further reinforced by targeting modified-ligands. For example, Zhan et al functionalized liposomes with amyloid-β-derived peptides. As a result, this functionalized system compared to non-targeting liposomes showed to have a higher BBB penetration efficacy, proved by in vitro models and in vivo biodistribution study. In the case of treating brain tumors though, the systems must be able to cross the BBB and target the brain tumor; thus, dual targeting strategy in liposome is a feasible strategy to achieve this. For example, research conducted by the Singh's Group modified the surface of transferrin and a cell-penetrating peptide, a modified-ligand. As a result, their experiment "showed around 12 and 3.3-fold increases of two chemotherapeutics (DOX and erlotinib) delivery, respectively compared to the free drugs" (Wu et al.) Therefore, this experiment proves that the dual strategy of liposomes greatly improves drug delivery for brain tumors. Interestingly though, liposomes without targeting-ligands can act as an external stimuli for brain drug delivery. For instance, Yanik et al. administered liposomes carrying drugs and through "applying two-component aggregation and uncaging focused ultrasound sequences at different stages," the drug soon uncaged itself from the lipid-vesicle and can cross the BBB without compromising the BBB's integrity. Overall, functionalized liposomal delivery systems present a promising prospective in efficiently achieving BBB drug delivery which is proven by multiple research experiments. However, more research needs to be conducted to further fine-tune the functionalization of brain-targeting liposomal delivery systems in order to be considered safe to be applied commercially.

Intranasal Administration

Intranasal administration provides a direct pathway for lipid-

drug delivery from the nasal cavity to the brain. There are multiple nose-to-brain pathways, but one of the most direct and often-studied routes is the olfactory nerve-olfactory bulb-brain. It offers the most shortest, direct and fastest route for drug-delivery, completing the delivery with a "time window of 1.5-6 h" (Wu et al., 2023). The nasal cavity of the nose is lined with a rich network of blood vessels which absorbs the drug into the olfactory pathway, extending from the nasal cavity to the olfactory bulb that is located near the brain (Tajes et al.). After that, the sensory neurons in the olfactory bulb absorb the drug where the it diffuses across the nasal mucosal barrier, the arachnoid membrane and then enters the stream of the cerebrospinal fluid (CSF) tracts to be absorbed into the bloodstream that extends to the CNS via CSF absorption by the superior sagittal sinus (Pardridge, Blood–Brain Barrier Delivery). This scenario was tested by Uchegbu et al. where they "constructed a nano-peptide with a 30–60 nm particle size, encapsulating leucine-enkephalin hydrochloride (LENK), and proved this nanoparticle was able to transport LENK through intranasal administration (Wu et al., 2023)." The results showed that LENK was found in the olfactory bulb. However, this method only works on lipid-soluble drugs that can diffuse across the two membranes: nasal mucosal barrier and the arachnoid membrane. Clinical trials tested on humans determined this method viable. However, the majority of CNS drugs are not lipid-soluble drugs or have MWs that are >400Da, which restricts their ability to freely diffuse across biological barriers; meaning that this delivery system only works on a small number of drugs. Nonetheless, drugs that cannot traverse across the nasal mucosal barrier can still enter the olfactory CSF but at the cost of inducing a local nasal injury. A human nose needs at least a volume >100 ml per nares to create a nasal injury, which results in the barrier breakdown of the nasal mucosal barrier. This finding is derived from the forced nasal administration of rats with large volumes such as 50ml per rat nare, causing a local nasal injury. Therefore, nasal injury could be a requirement in order for water-soluble drugs to travel from the nasal cavity and into the olfactory CSF successfully. Furthermore, the portion of the nasal mucosa that is olfactory in the rat is 50% of the total surface area of the nose; meanwhile in humans, the olfactory part of the nasal mucosa is only 3–8%. Thus, it can be presumed that when administering a volume that is not injurious to the nose, the drug would not be distributed into the CSF; this scenario has been observed when inserting small molecules such as melatonin and vitamin B12 (Pardridge, Blood–Brain Barrier Delivery). Additionally, other limitations of this drug delivery system are the differences in the shape of patients' nasal cavities, the precise dosing of the drug that is needed, the mucociliary elimination, and drainage to the pharynx and to lower parts of the body. As well as other physiological obstacles such as nasal pathogens, a higher pH, a higher enzymatic activity of the epithelium, and mucosal irritation that can all hinder the efficacy and execution of this method (Tajes et al.). In conclusion, intranasal drug delivery contains limiting factors that still makes the approach unfeasible despite its promising potential.

RESULT

Article	Focus	Major findings
Inflammatory Mediators and Modulation of Blood Author: N. Joan Abbott Year published: 1998	The effects of histamine, bradykinin, nucleotides, and serotonin on the blood-brain barrier's permeability.	All stimuli demonstrated BBB opening but via different ways given their unique interactions with the BBB's structure and an involvement of cAMP and nitric oxide in BBB disruption.
Regulation of Blood–Brain Barrier Permeability Author: William G. Mayhan Year published: 2003	Examining BBB permeability under pathophysiological conditions.	BBB permeability is effectively increased when under inflammation as these elements inflict opening of the BBB and reduction in electrical resistance.
The blood-brain barrier: Bottleneck in brain drug development Author: William M. Pardridge Year published: 2005	Evaluates existing prominent methods in BBB drug delivery or opening.	Many of the methods such as intranasal administration, lipidization of molecules, and other forms of invasive and non-invasive methods all result in BBB opening but still carry adverse effects that require further refining of the technique.
Modern methods for delivery of drugs across the blood–brain barrier Authors: Yan Chen, Lihong Liu Year published: 2012	Explains the obstacles attributing the BBB issue and existing methods of drug transport in BBB.	Many of the methods such as intranasal administration, lipidization of molecules, and other forms of invasive and non-invasive methods all result in BBB opening but still carry adverse effects that require further refining of the technique.

The blood-brain barrier: Structure, function and therapeutic approaches to cross it Authors: Marta Tajes, Eva Ramos-Fernández, Xian Weng-Jiang, Mònica Bosch-Morató, Biuse Guivernau, Abel Eraso-Pichot, Bertrán Salvador, Xavier Fernàndez-Busquets, Jaume Roquer Francisco J. Muñoz Year published: 2013	Explains the structure, function, and existing methods of drug transport in BBB.	Many of the methods such as intranasal administration, lipidization of molecules, and other forms of invasive and non-invasive methods all result in BBB opening but still carry adverse effects that require further refining of the technique.
The blood–brain barrier: structure, regulation, and drug delivery Authors: Di Wu, Qi Chen, Xiaojie Chen, Feng Han, Zhong Chen & Yi Wang Year published: 2023	Explains the structure, function, and existing methods of drug transport in BBB.	Many of the methods such as intranasal administration, lipidization of molecules, and other forms of invasive and non-invasive methods all result in BBB opening but still carry adverse effects that require further refining of the technique.
Blood-brain barrier delivery Author: William M. Pardridge Year published: 2007	Explains the structure, function, and existing methods of drug transport in BBB.	Many of the methods such as intranasal administration, lipidization of molecules, and other forms of invasive and non-invasive methods all result in BBB opening but still carry adverse effects that require further refining of the technique.

DISCUSSION

When inflicting an inflammation inside the BBB, unknown mechanisms and other processes that also contribute to BBB permeability such as nitric oxide, cytokines and other second messengers need to be studied of their conflicting roles. Should researchers grasp their function, it can provide valuable insights to manipulate these components to further make drug delivery more effective when under a pathophysiological condition. As for hyperosmotic agents, they possess harmful effects to neurons that need to be investigated to be able to mitigate these damages. Although externally-applied stimuli such as ultrasounds are invasive techniques, their harmful effects are less compared to the other two; although it shows modest opening of the BBB, researchers need to inquire how to safely let ultrasound pulses concentrate within a tissue without risking damage to its surrounding tissue. Furthermore, the pursuit of the lipidization of water-soluble drugs is greatly hindered due to the limits of medicinal chemistry. This goal requires an innovative breakthrough within medicinal chemistry to push the boundaries of these drugs in order to fit within the MW and hydrogen bonding rules; thus, it is uncertain if researchers would be able to find a solution to this issue. Meanwhile, liposome drug delivery can be a potential method; scientists need to advance this technique, focusing on the functionalization of liposome drug delivery to enhance its performance or enable unique functions and interactions with the BBB to permit the liposome across the BBB.

CONCLUSION

BBB disruption techniques discussed in this research paper demonstrated efficacy in BBB opening but also harmful side effects. The opening of the BBB simultaneously lets harmful molecules, pathogens and other unwanted substances into the brain's sensitive environment. Therefore, it is imperative for researchers when performing BBB disruption techniques to have another system implemented that allows them to control the opening of the BBB and ensures that the process is reversible and temporary. Despite multiple research discrepancies and uncertainties shown in almost all BBB techniques, it is obvious that the BBB does increase in permeability when undergoing an inflammatory process; although this method still requires further research and understanding of the phenomena occurring in the BBB. On the other hand, non-invasive BBB techniques are known for their harmless approach. Compared to all of the other techniques discussed, liposome drug delivery systems showed the greatest potential in becoming a plausible solution to the BBB for patients. Although all of these techniques still possess uncertainties and incomprehension, studies dedicated to liposomes have reinforced that it is the most versatile and efficient method of drug delivery transport, encapsulating both hydrophobic and hydrophilic drugs that cannot pass the BBB. With further development of liposome drug delivery, they display a promise in becoming the next solution to BBB drug delivery. Conversely though, intranasal administration of drugs may pose an uncertain method as it possesses multiple physiological obstacles that hinder drug distribution in the brain such as differences in nasal structures, potential allergies, drainage and drug elimination, and etc. In conclusion, it is obvious that all of these methods still require further development to minimize their harmful side effects and research to understand unknown mechanisms involving BBB permeability, but the future shows a guarantee that there will definitely be a solution in bypassing the BBB to finally cure brain diseases.

REFERENCES

1. Chen, Yan, and Lihong Liu. Modern Methods for Delivery of Drugs across the Blood–Brain Barrier. Vol. 64, no. 7, 1 May 2012, pp. 640–665, www.sciencedirect.com/science/article/pii/S0169409X11002900, https://doi.org/10.1016/j.addr.2011.11.010.
2. G. MAYHAN, WILLIAM . "Regulation of Blood–Brain Barrier

Permeability." Microcirculation, 2013, www.tandfonline.com/doi/abs/10.1080/mic.8.2.89.104.
3. N. Joan Abbott. Inflammatory Mediators and Modulation of Blood–Brain Barrier Permeability. Vol. 20, no. 2, 1 Jan. 2000, pp. 131–147, link.springer.com/article/10.1023/A:1007074420772, https://doi.org/10.1023/a:1007074420772. Accessed 5 July 2023.
4. Pardridge, William M. Blood–Brain Barrier Delivery. Vol. 12, no. 1-2, 1 Jan. 2007, pp. 54–61, www.sciencedirect.com/science/article/abs/pii/S1359644606004363, https://doi.org/10.1016/j.drudis.2006.10.013.
5. ---. The Blood-Brain Barrier: Bottleneck in Brain Drug Development. Vol. 2, no. 1, 1 Jan. 2005, pp. 3–14, link.springer.com/article/10.1602/neurorx.2.1.3, https://doi.org/10.1602/neurorx.2.1.3.
6. Tajes, Marta, et al. "The Blood-Brain Barrier: Structure, Function and Therapeutic Approaches to Cross It." Molecular Membrane Biology, 2014, www.tandfonline.com/doi/full/10.3109/09687688.2014.937468
7. Wu, Di, et al. The Blood–Brain Barrier: Structure, Regulation, and Drug Delivery. Vol. 8, no. 1, 25 May 2023, www.nature.com/articles/s41392-023-01481-w#Abs1, https://doi.org/10.1038/s41392-023-01481-w.